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Photocrosslinked maleilated chitosan/methacrylated poly (vinyl alcohol) bicomponent nanofibrous scaffolds for use as potential wound dressings

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ABSTRACT

To improve water stability of hydrophilic nanofibers, photocrosslinked maleilated chitosan/methacrylated poly (vinyl alcohol) (MCS/MPVA) bicomponent nanofibrous scaffolds were successfully obtained by electrospinning of aqueous MCS/MPVA solution and consequent photopolymerization. The parameters of MCS/MPVA solutions such as viscosity and conductivity were measured to evaluate electrospinnability of the blend solutions. The bicomponent nanofibrous scaffolds were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis and differential scanning calorimetry (DSC), respectively. SEM results indicated that MCS/MPVA weight ratios significantly influenced the morphology and diameter distribution of the nanofibers. XRD and DSC investigated that there was strong interaction caused by hydrogen bonding between molecular chain of MCS and MPVA. Water stability test confirmed that the photocrosslinked matrix with a MCS/MPVA ratio of 10/90 retained excellent integrity of the fibrous structure in water. The in vitro cytotoxicity evaluation revealed that photocrosslinked nanofibrous scaffolds entailed good cellular compatibility, and could be used as potential wound dressing.

1. Introduction

Serious skin damage such as full-thickness burns or deep ulcers always needs skin grafts. However, many skin substitutes for grafts have been restrictively employed due to their disadvantages such as high cost, the limited availability of self skin grafts, and problems of immune response and disease transmission (MacNeil, 2007; Seal, Otero, & Panitch, 2001; Vacanti, Langer, Upton, & Marler, 1998). Thus, many tissue engineered skin substitutes get developed to resolve all the issues mentioned above. Hereinto, nanofiber matrices have shown tremendous promise, because of their unique properties such as oxygen-permeable high porosity, variable pore-size distribution, high surface to volume ratio, and so on. Particularly, their morphology show high similarity to that of the natural extracellular matrix in skin, promoting cell adhesion migration and proliferation (Courtney Sacks, Stankus, Guan, & Wagner, 2006; Rosic et al., 2013; Xu et al., 2009). Electrospinning represents a relatively simple way of creating polymer nanofibers for wound healing applications (Jeong et al., 2011; Ma et al., 2012; Shalumon et al., 2010). Hereinto, chitosan (CS) is one of the most promising biomacromolecules for wound dressing, due to its favorable excellent biological contributions to the wound, such as biodegradability, biocompatibility, and antibacterial activity (Khor & Lim, 2003; No, Park, Lee, & Meyers, 2002; Ueno, Mori, & Fujinaga, 2001) as well as wound healing acceleration (Klossner, Queen, Coughlin, & Krause, 2008). Meanwhile, poly (vinyl alcohol) (PVA) is of particular interest for wound dressing, attributed to its nontoxicity and biocompatibility (Ignatova, Starbova, Markova, Manolova, & Rashkov, 2006; Millon, Guhados, & Wan, 2008). Additionally, PVA is often used to aid natural polymers to get nanofibers with overcoming electrospinning limitations, due to its excellent electrospinnability (Jannesari, Varshosaz, Morshed, & Zamani, 2011). In the past few years, electrospun CS/PVA composite nanofibers have been successfully fabricated (Li & Hsieh, 2006; Zhou, Yang, & Nie, 2006). However, some acids solvents such as acrylic acid, formic acid and acetic acid must be employed during the fabrication of these composite nanofibers. The residue of toxic acid solvent in electrospun products is detrimental when it is applied to wounded human skin or tissue. In addition, these water-soluble fibrous mats should be chemically crosslinked to prevent rapid hydrolysis of the

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nanofibers in aqueous media, which is another major drawback of but always desirable for tissue engineering applications. However, the commonly-used crosslinking agents are cytotoxic agents such as glutaraldehyde (Hang, Tae, & Park, 2010; Mi, Vijayaragavan, & Heldt, 2014; Sundaramurthi, Vasanthan, Kuppan, Krishnan, & Sethuraman, 2012). Thermal treatment is a well-known technology to initiate the crosslinking reaction, accordingly resulting in high temperatures and making damage to incorporated bioactive factors and cells (Du & Hsieh, 2008).

Herein, in order to resolve these issues, water-soluble maleilated chitosan was synthesized under mild and heterogeneous reaction conditions without using the acid as a solvent. And the carboxyl group in maleilated chitosan could also offer the binding site to bioactive nitric oxide derivative (Jokhadze, Machaidze, Panosyan, Chu, & Katsarava, 2007; Zhong, Wu, Reinhart-King, & Chu, 2010), which could help to maintain an effective level of nitric oxide in the wound for promotion of wound healing (Luo & Chen, 2005) when it was used as a wound dressing. Meanwhile, methacrylated poly (vinyl alcohol) was synthesized, and thus photocrosslinked maleilated chitosan/methacrylated poly (vinyl alcohol) bicomponent nanofibrous scaffolds prepared with UV irradiation. Here, water is a good solvent for both fabrication processes and biomedical applications of MCS and MPVA, and electrospinning carried out in neutral pH and further photocrosslinking could not only avoid the trace presence of the toxic solvent or crosslinking agent, but also result in the fabrication of functional fibrous biomedical products containing thermo-sensitive protein drugs or cells. The morphological characterization, crystallization and water resistance were further investigated. The in vitro cytotoxic evaluation of this nanofiber in combination with mouse fibroblasts (L929) was finally measured to indicate its potential for a scaffolding material for skin.

2. Experimental

2.1. Materials

Chitosan (CS, viscosity = 80 mPa s, degree of deacetylation = 93.7%) was purchased from Jinhu Crust Product Co., Ltd., China. Viscosity-average molecular weight of chitosan determined by viscometer was 1.02×10^6. And the degree of deacetylation of chitosan was calculated as 84.5% by ^{1}H NMR. Poly (vinyl alcohol) (Number-average molecular weight = 1.7×10^5, 88% hydrolyzed) was obtained from Kuraray Co, Ltd., Japan. Maleic anhydride (MA) and 4-Dimethylaminopyridine (DMAP) was purchased by Sinopharm Chemical Reagent Co, Ltd. Glycidyl Methacrylate (GMA) was supplied by Adamas Reagent Co., Ltd. Cytocompatible UV photoinitiator Darocur 2959 (D-2959, 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone) was donated from Ciba-Geigy Chemical Co. (Tom River, NJ) (Williams, Malik, Kim, Manson, & Elisseeff, 2005). Mouse fibroblasts (L929) were purchased from Wuhan Beinglay Biological Technology Co., Ltd., China. Dulbecco's modified eagle medium (DMEM), 1% Penicillin-streptomycin, trypsin, 10% fetal bovine serum (FBS), MTT powder was supplied by Shanghaikayon Biological Technology Co., Ltd., China. Other reagents were all A.R. grade.

2.2. Synthesis of maleilated chitosan (MCS)

In order to provide reactive sites on chitosan molecules for further photopolymerization, a modified chitosan carrying vinyl carboxylic acid groups was designed and synthesized. Briefly, 1.0 g of chitosan and maleic anhydride (3.5 g) was added into in 100 mL of dimethyl sulfoxide (DMSO). The mixed solution was allowed to stay for 24 h at 60 °C. After that, saturated $NaHCO_3$ solution was added to the reaction mixture to adjust the pH to 8–9, after which the mixture was dialyzed against water for 2 days and lyophilized to obtain pure MCS. A Bruker AV 400 NMR instrument was used to characterize ^{1}H NMR spectrum.

2.3. Synthesis of methacrylated poly (vinyl alcohol) (MPVA)

MPVA was prepared according to the method reported by Hennink et al. (Dijk-Wolthuis et al., 1995). Briefly, 5.0 g PVA was dissolved in 100 mL dimethyl sulfoxide (DMSO) and DMAP was added to it at 1.0 mol% relative to the hydroxyl group of PVA. 0.64 g of GMA was added in molar ratio to hydroxyl group of PVA and the reaction mixture was stirred for 6 h at 60 °C. The mixture was precipitated by acetone and then dried under vacuum for 2 days and stored at −5 °C in the dark. ^{1}H NMR spectrum was recorded on a Bruker AV 400 NMR instrument.

2.4. Preparation of polymer solutions

A 5% (w/v) MCS solution was prepared by dissolution of 5.0 g MCS in 100 mL distilled water. MPVA (10.0 g) was dissolved in 100 mL distilled water to form a MPVA solution at a concentration of 10% (w/v). The MCS solution was mixed with the MPVA solution at MCS/MPVA weight ratio of 100/0, 75/25, 50/50, 25/75, 20/80, 10/90 and 0/100, respectively.

The conductivities of the MCS/MPVA solutions were measured by electric conductivity meter (DDB-6200, Shanghai Rex Xinjing Instrument Co. Ltd., China), and the viscosities of the blend solutions were performed as shear rate swept from 0.01 to 1000 s^{-1} on an AR 2000ex rheometer (TA Instrument, USA) using a 40 mm steel parallel plate geometry and 1 mm gap at 25 °C.

2.5. Preparation of fiber mats

The electrospinning set-up was composed of a 20 mL plastic syringe with stainless needle (0.57 mm inner diameter), a grounded aluminum foil as the collector and a high voltage power supply (EST705, Beijing Huajinghui Technology Co., Ltd., China). 25 kV dc voltage and 12 cm distance was applied between needle and plate. And the solution flow rate was 1.0 mL/h.

2.6. Photocrosslinking of the electrospun membranes

For further photocrosslinking to improve water stability, the nanofiber mats were prepared from electrospinning of MCS/MPVA solution (MCS/MPVA = 10/90) with the photoinitiator D-2959 (0.1 w/v%) and then directly irradiated under 50 W Hg lamp at exposure intensity of 30 mW/cm^2. The crosslinked nanofibers were dried in vacuum at 25 °C for 12 h.

2.7. Characterizations

2.7.1. Scanning electron microscopy (SEM)

The morphology and diameter of nanofibrous mats were determined by scanning electron microscope (JSM-6510, JEOL Ltd., Japan) at accelerating voltage of 10 kV. The diameters of nanofibers were measured by using image analyzer (Image J, version 1.37 v, National Institutes of Health, USA). At least thirty fibers were statistic in image.

2.7.2. Differential scanning calorimetry (DSC)

DSC studies were performed on a DSC822e differential scanning calorimetric analyzer under nitrogen atmosphere, at a flow rate of 50 mL/min. The samples were heated from −50 °C to 220 °C at a scanning rate of 10 °C/min using aluminum pans.